IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	)	Examiner: Kemmerer, Elizabeth
Avi Ashkenazi, et al.	)	Art Unit: 1646
Application Serial No. 09/904,766	)	Confirmation No. 4054
Filed: July 12, 2001	)	Attorney's Docket No. 39780-1618P1C33
For: **PRO269 POLYPEPTIDES**	)	**Customer No. 35489**

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ON APPEAL TO THE BOARD OF PATENT APPEALS AND INTERFERENCES
APPELLANTS' REPLY BRIEF

MAIL STOP APPEAL BRIEF - PATENTS
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

On January 13, 2005, the Examiner made a final rejection to pending Claims 44-46 and 49-52. A Notice of Appeal was filed on June 13, 2005, and Appellants' Appeal Brief was filed September 13, 2005.

An Examiner's Answer was mailed on November 15, 2005. The following constitutes Appellants' Reply Brief in response to the Examiner's Answer and is timely filed (January 15 and 16, 2006 being a Sunday and a Federal Holiday in the District of Columbia, respectively). This Reply Brief is accompanied by a Request for Oral Hearing.

Appellants have filed concurrently a Petition for Designation as New Grounds of Rejection Under 37 C.F.R. §1.181. A copy is enclosed herewith.

ARGUMENTS

Claim Rejections Under 35 U.S.C. §101 and § 112, first paragraph

Concerning the rejection of Claims 44-46 and 49-52 under 35 U.S.C. §101 as allegedly lacking a specific, substantial and credible asserted utility or a well established utility. The Examiner cites the following arguments and cites new references in support of these conclusions.

(1) The genomic DNA encoding PRO269 had a ΔCt value of at least 1.0 for eight out of seventeen lung tumor samples. Genomic DNA encoding PRO269 was not amplified in any of the seventeen colon tumor samples (Examiner's Answer, page 4 –5).

(2) In order for PRO269 polypeptides to be overexpressed in lung tumors, amplified genomic DNA would have to correlate with amplified mRNA. The art allegedly discloses that such correlations cannot be presumed. (Examiner's Answer page 5). The Examiner cites Pennica *et al.*, and Konopka *et al.*

(3) In order for PRO269 polypeptides to be overexpressed in lung tumors, amplified mRNA would have to correlate with amplified polypeptide. (Examiner's Answer, page 5). The art allegedly discloses that such correlations cannot be presumed. The Examiner cites Hu et al (2003), Haynes et al., (1999) and **new references** LaBaer (2003), Gygi *et al.* (1999), Chen *et al.* (2002), Lian *et al.* (2001), Fessler *et al.* (2002) and Greenbaum (2003) for support.

The same arguments are cited in support of the rejection of claims 44-46 and 49-52 under 35 U.S.C. §112, first paragraph, for alleged lack of enablement for how to use the invention, since the claimed invention is allegedly not supported by either a credible, specific and substantial asserted utility or a well established utility.

Appellants disagree with each of the Examiner's arguments on a number of grounds.

1. New Grounds of Rejection have been made by the Examiner

First Appellants note that the Examiner has raised six new references for the first time in the Examiner's response. They are:

(1) Chen *et al.*; 2002, Molecular and Cellular Proteomics 1:304-313;

(2) LaBaer; 2003, Nature Biotechnology 21:976-977;

(3) Gygi *et al.*, 1999, Mol. Cell. Biol. 19:1720-1730;

(4) Lian *et al.* 2001, Blood 98:513-524;

(5) Fessler *et al*, 2002, J. Biol. Chem. 277:31291-31302; and

(6) Greenbaum et al., 2003, Genome Biology 4:117.1-117.8.

These references were not previously cited in any of the prior rejections of record. Appellants submit that the citation of such new prior art references for the first time in an Examiner's answer constitutes a new ground of rejection and is not permissible.

The M.P.E.P. Section 1207.03 (III) states that:

> A new prior art reference cited for the first time in an Examiner's answer generally will constitute a new ground of rejection. If the citation of a new prior art reference is necessary to support a rejection, it must be included in the statement of rejection, which would be considered to introduce a new ground of rejection. Even if the prior art reference is cited to support the rejection in a minor capacity, it should be positively included in the statement of rejection. *In re Hoch*, 428 F.2d 1341, 1342 n.3, 166 USPQ 406, 407 n. 3 (CCPA 1970). However, where a newly cited reference is added merely as evidence of the prior well known statement made by the examiner, the citation of the reference in the Examiner's answer would not constitute a new ground of rejection within the meaning of 37 C.F.R. §1.192(a)(2). See also M.P.E.P. §2144.03.

The M.P.E.P. adds that:

> In addition, if an Appellant has clearly set forth an argument in a previous reply during prosecution of the application and the Examiner has failed to address that argument, the Examiner would not be permitted to add a new ground of rejection in the Examiner's answer to respond to that argument but would be permitted to

reopen prosecution, if appropriate. (Emphasis added; See M.P.E.P. §1207.03; Requirements for a new ground of rejection, II).

The Court of Customs and Patent Appeals considered this situation in *In re Hoch*, 428 F.2d 1341, 1342 n.3, 166 USPQ 406, 407 n. 3 (CCPA 1970). In that case there were two other references cited in the appeal that were not mentioned in the statement of either of the appealed rejections. The court held:

> Appellant's complaint seems to be justified, and if we did not find the rejections based *solely* on Molotsky and the French patent to be sound, we might well feel constrained to reverse the decision of the board. Where a reference is relied on to support a rejection, whether or not in a "minor capacity" there would appear to be no excuse for not positively including the reference in the statement of rejection.

Appellants note that a Reply Brief must be in compliance with the requirements set forth in 37 C.F.R. §41.41. New or non-admitted affidavits and/or other evidence are not permitted in a Reply Brief.

For the reasons set forth in their Petition, Appellants submit that the citation for the first time of these six references constitutes new grounds of rejection and accordingly such rejections are not permissible.

Appellants have filed a Petition herewith which requests that the grounds of rejection and the six new references that are being cited in the Examiner's Answer in support of the grounds of rejection be designated new grounds of rejection. Appellants request a corrected Examiner's Answer that identifies the rejections as new grounds for rejection. Appellants request the prosecution be reopened.

2. Reply to the Examiner's Arguments.

The Examiner's arguments will be addressed in the order they are listed above.

(1) In making the rejection that "only eight out of seventeen lung tumor samples tested positive" and "PRO269 was not amplified in any of the seventeen **colon** tumor samples" (emphasis added) (Examiner's Answer, pages 4-5), the Examiner seems to indicate that a tumor marker is patentable only if the marker tests positive in a statistically high number of samples compared to

the total number of samples tested or if the tumor tests positive in every tissue type that was studied. However, this is not legally correct. Neither the M.P.E.P. nor the Utility Guidelines require that it is necessary for the Appellant to show a positive result in most or a larger percentage of the tissue samples studied in order to make an assertion of utility, nor are they needed to show that the tumor marker identifies cancers of various tissues types, *e.g.*: lung, colon, etc. The above remarks by the Examiner are a clear indication that the Examiner applies a standard that might be appropriate, if the issue at hand were the regulatory approval of a diagnostic assay based on the overexpression of PRO269 in lung tumor, but is fully inappropriate for determining if the "utility" standard of the Patent Statute is met. The FDA reviewing an application for a new diagnostic assay will indeed ask for actual numerical data, statistical analysis, and other specific information before a diagnostic assay is approved. However, the Patent and Trademark Office is not the FDA, and the standards of patentability are not the same as the standards for market approval. It is well established law that therapeutic utility sufficient under the patent laws is not to be confused with the requirements of the FDA with regard to safety and efficacy of drugs to be marketed in the United States. *Scott v. Finney*, 34 F.3d 1058, 1063, 32 USPQ2d 1115, 1120 (Fed. Cir. 1994). Indeed, in *Nelson v. Bowler*, 626 F.2d 853, 856, 206 USPQ 881, 883 (CCPA 1980), the Federal Circuit found that the identification of a pharmacological activity of a compound provides an "immediate benefit to the public" and satisfies the utility requirement. This logically applies to a diagnostic utility as well. The identification of a diagnostic utility for a compound should suffice to establish an "immediate benefit to the public" and thus to establish patentable utility.

Furthermore, as indicated previously, it is well-accepted in the art that not all tumor markers are generally associated with every tumor, or even, with most tumors. In fact, some tumor markers are useful for identifying rare malignancies. That is, even if the association of a tumor marker with a particular type of tumor lesion is rare, or, even if the occurrence of a particular kind of tumor lesion itself is rare, since such markers identifying rare tumors, they have great value in tumor diagnosis, and consequently, in tumor prognosis. The ΔCt values for

PRO269 of at least 1-2 ΔCt units, which correspond to $2^{1.04}$ -$2^{1.80}$- fold amplification or 2.056 to 3.482 fold amplification in primary lung tumors, were considered significant according to the Goddard declaration. The skilled artisan would know the value and utility of rare tumor markers. Further, Appellants need not show that DNA was amplified in colon tumors as well for an assertion of utility.

(2) In support of the assertion that there is "a poor correlation between gene amplification and mRNA expression," the Examiner cites Pennica *et al.*, Konopka *et al.* (Examiner's Answer pages 5, 10, 14, and 18)

References Pennica and Konopka were discussed previously in the Appeal Brief filed September 15, 2005 and Appellants maintain, for the reasons set forth therein, that they cannot be used to establish a poor correlation between mRNA and protein because these references did not show that, in general, it is more likely than not for mRNA and protein levels not to have a correlation. The reasons were clearly discussed in the Appeal Brief. Accordingly, the Examiner has not met her burden of proof.

The Examiner cited Pennica *et al.* as providing general evidence showing lack of correlation between gene (DNA) amplification and elevated mRNA levels. (Examiner's Answer, pages 5, 10, 14 and 18). The standard, however, is not absolute certainty. The fact that in the case of a specific class of closely related molecules there seemed to be no correlation with gene amplification and the level of mRNA/protein expression, does not establish that it is more likely than not, in general, that such correlation does not exist. The Examiner has not shown whether the lack of correlation observed for the family of WISP polypeptides is typical, or is merely a discrepancy, an exception to the rule of correlation. Indeed, the working hypothesis among those skilled in the art is that, if a gene is amplified in cancer, the encoded protein is likely to be expressed at an elevated level. In fact, as noted even in Pennica *et al.*, "[a]n analysis of *WISP*-1 gene amplification and expression in human colon tumors *showed a correlation between DNA amplification and over-expression* . . . ." (Pennica *et al.*, page14722, left column, first full

paragraph, emphasis added). Accordingly, Appellants respectfully submit that Pennica *et al.* teaches nothing conclusive regarding the absence of correlation between amplification of a gene and over-expression of the encoded WISP polypeptide. More importantly, the teaching of Pennica *et al.* is specific to *WISP* genes in human colon tumors. Pennica *et al* has no teaching about lung tumors. Pennica *et al.* has no teaching whatsoever about the correlation of gene amplification and protein expression in general.

The Examiner cited Konopka *et al.* to establish that protein expression is generally not related to gene amplification. (Examiner's answer, pages 5, 10, 14 and 18). Appellants submit that the PTO has generalized a very specific result disclosed by Konopka *et al.* to cover all genes. Konopka *et al.* actually state that "[p]rotein expression is not related to amplification of the ***abl*** gene but to variation in the level of *bcr-abl* mRNA produced from a single Ph^1 template." (See Konopka *et al.*, Abstract, emphasis added). The paper does not teach anything whatsoever about the correlation of protein expression and gene amplification in general, and provides no basis for the generalization that apparently underlies the present rejection. The statement of Konopka *et al.* that "[p]rotein expression is not related to amplification of the *abl* gene . . ." is not sufficient to establish a *prima facie* case of lack of utility. More importantly, the teaching of Konopka *et al.* is specific to the abl gene in B-lymphoid cell lines from chronic myelogenous leukemia patients. Konopka *et al.* has no teaching about lung tumor cells. It is not enough to show that for a particular gene a correlation does not exist. The law requires that the Examiner show evidence that it is more likely than not that such correlation, in general, does not exist. Such a showing has not been made.

The Examiner argues that Pennica *et al* and Konopka *et al.* "are relevant even though they are not reviews of gene amplification for genes in general because they show a lack of correlation" and the instant case also concerns a single gene. (Examiner's Answer, page 18) Appellants disagree. The test is whether it is more likely than not that gene amplification results in overexpression of the mRNA of the gene. In order to meet that standard, the Examiner must provide evidence that it is more likely than not that gene amplification does not result in

overexpression. The Examiner has agreed that these references do not provide a review of the correlation of gene amplification and overexpression in general. Accordingly, Appellants maintain that the Examiner has not met the burden.

On the other hand, Appellants have submitted ample evidence to show that, in general, if a gene is amplified in cancer, it is more likely than not that the encoded protein will be expressed at an elevated level. First, the articles by Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.*, (made of record in Appellants' Response filed November 3, 2004) collectively teach that in general, gene amplification increases mRNA expression.

For example, Orntoft *et al.* studied transcript levels of 5600 genes in malignant bladder cancers many of which were linked to the gain or loss of chromosomal material using an array-based method. Orntoft *et al.* showed that there was a gene dosage effect and taught that "in general (18 of 23 cases) chromosomal areas with more than 2-fold gain of DNA showed a corresponding increase in mRNA transcripts" (Column 1, abstract).

The Examiner criticizes Orntoft *et al.* on the basis that Orntoft *et al.* concentrated on regions of chromosomes with strong gains of chromosomal material containing clusters of genes. Accordingly, Orntoft *et al.*'s finding could allegedly only be extended to other genes in such clusters and this analysis was not done for PRO269. Therefore the findings of Orntoft *et al.* cannot be extended to PRO269. (Examiner's Answer, page 23) Appellants fail to see how this argument is pertinent. Orntoft *et al.* is a review of the correlation of gene amplification and mRNA expression.

The Examiner criticizes Orntoft *et al.* on the basis that Orntoft *et al.* compared genes from non-invasive transitional cell carcinomas to genes from invasive transitional cell carcinomas. There allegedly was no comparison between genes in cancerous versus non-cancerous tissue. Further Orntoft *et al.*, did not study lung cancer. Appellants note that Orntoft *et al.* state that it was a strength of the investigation that they were able to compare invasive tumors to benign tumors rather than to normal urothelium, as the tumors studied were biologically very close and probably represent successive steps in the progression of bladder

cancer. (page 44). Accordingly, the identification of the correlation by Orntoft *et al.* of a correlation between gene amplification and mRNA overexpression is more meaningful. Furthermore, it is improper for the Examiner to criticize Orntoft *et al.* for not referring to lung tumors when neither of the references supplied by the Examiner refer to lung tumors.

The Examiner criticizes the specification on the basis that the specification allegedly discloses low levels of amplification of DNA. (Examiner's Answer, page 24) Appellants note that Orntoft *et al.* states that chromosomal areas with more than a 2-fold gain in DNA showed a corresponding increase in mRNA transcripts. (abstract) Appellants note that they have shown a more than 2-fold amplification of PRO269 DNA in Example 92.

In addition, Hyman *et al.* showed, using CGH analysis on cDNA microarrays which compared DNA copy numbers and mRNA expression of over 12,000 genes in breast cancer tumors and cell lines, that there was "evidence of a prominent global influence of copy number changes on gene expression levels." (Page 6244, column 1, last paragraph).

The Examiner criticizes Hyman *et al.* on the basis that Hyman allegedly found 44% (less than half) of *highly* amplified genes showed overexpression at the mRNA level and 10.5% of *highly* overexpressed genes were amplified. (Examiner's Answer, page 24)

First Appellant notes that the Examiner has not completely quoted the disclosure of Hyman *et al.* Hyman states that "[u]p to 44% of the highly amplified transcripts (CGH >2.5) were overexpressed (i.e. belonged to the global upper 7% of expression ratios), compared with only 6% for genes with normal copy number levels. (Fig. 1A). Conversely, 10.5% of the transcripts with high-level expression (cDNA ratio, >10) showed increased copy number (Fig. 10B). Low-level copy number increases and decreases were also associated with similar, although less dramatic outcomes on gene expression." (page 6242). In conclusion, Hyman states "This analysis provided: (a) evidence of a prominent global influence of copy number changes on gene expression levels." (page 6244). Accordingly, this criticism of Hyman is misplaced and Hyman shows that it is more likely than not that gene amplification correlates with mRNA expression.

Secondly the Examiner states that Hyman *et al* shows that only 10.5% of transcripts with high-level expression showed increased copy number. This number is not pertinent to the present case. Appellants are not alleging that all increases in mRNA expression are related to gene amplification. It is acknowledged that there are other reasons for increased mRNA expression. Appellants are simply stating that where there is gene amplification it is more likely than not that there is increased mRNA expression.

Thirdly, the Examiner alleges that Hyman at page 6244 states that of the 12,000 transcripts analyzed, a set of 270 was identified in which overexpression was attributable to gene amplification. This proportion is allegedly approximately 2%. The Examiner maintains that 2% does not provide a reasonable expectation that a slight amplification of PRO269 would be correlated with overexpression. Appellants note that here again the Examiner is considering the wrong percentage. The Examiner is determining the percentage of genes in the genome that are amplified and overexpressed. Appellant's argument is not that a majority of the genes in the genome are amplified and overexpressed. Appellant's position is that if a gene is amplified, it is more likely than not that the gene product is overexpressed. This is exactly what Hyman teaches.

Additional supportive teachings were also provided by Pollack *et al.,* who studied a series of primary human breast tumors and showed that "62% of highly amplified genes show moderately or highly elevated expression, and DNA copy number influences gene expression across a wide range of DNA copy number alterations (deletion, low-, mid- and high-level amplification), and that on average, a 2-fold change in DNA copy number is associated with a corresponding 1.5-fold change in mRNA levels." (emphasis added)

The Examiner criticizes Pollack on the basis that Pollack concentrated on large chromosome regions showing high amplification (p.12965). Further Pollack is allegedly limited to highly amplified genes which were not evaluated by the method of the instant specification and did not test for protein expression levels. Also Pollack did not study lung cancer. (Examiner's Answer, page 25 – 26)

Appellants disagree with the criticism of Pollack for the following reasons. First, Appellants can find no disclosure in Pollack which indicates that it is limited to regions showing high amplification. In Pollack, test DNA was labeled and hybridized to a human cDNA microarray containing 6,691 different mapped human genes (i.e. Unigene clusters) (page 12963). Pollack states that their method had the sensitivity to detect 1.5, 2 or 2.5 fold gains in single copy DNA. (page 12964). Finally, it is improper for the Examiner to criticize Pollack as being irrelevant because it is not directed to lung tumors, when the references provided by the Examiner are similarly not directed to lung tumors.

Thus, these articles, Orntoft, Hyman and Pollack collectively teach that in general, gene amplification increases mRNA expression.

(3) In support of the assertion that even if increased mRNA levels could be established for PRO269, it does not follow that polypeptide levels would also be amplified, the Examiner cites Chen *et al.* **(newly cited)**, LaBaer **(newly cited),** Hu *et al.*, Haynes *et al.,* Gygi *et al.* **(newly cited)**, Lian et al. **(newly cited)**, Fessler *et al.*, **(newly cited)**, and Greenbaum *et al.*, **(newly cited)**. (Examiner's Answer, pages 6 – 7, 10, 11, 14, and 17). The Examiner divides these references into two groups: evidence that mRNA does not correlate with increased polypeptide levels in healthy tissue (Haynes *et al.*, Gygi *et al.*, Lian *et al.* and Fessler *et al.*) or cancerous tissue (see Hu *et al.*, LaBaer, Chen *et al.,* and Hanna *et al.*) (Examiner's Answer page 29)

The Examiner repeatedly offers Hu *et al.* as allegedly analyzing 2286 genes that showed a greater than 1-fold difference in mean expression level between breast cancer samples and normal samples in a microarray. Hu *et al.* allegedly discovered that for genes displaying a 5-fold change or less in tumors compared to normal, there was no evidence of a correlation between altered gene expression and a known role in the disease. However, among genes with a 10-fold or more change in expression level, there was allegedly a strong and significant correlation between expression level and a published role in the disease. (Examiner's Answer, pages 6, 14-15, 20, 27 and 36) The Examiner states that based on Hu *et al.* the skilled artisan allegedly

would not reasonably expect PRO269 protein can be used as a cancer diagnostic. (Examiner's Answer page 31)

In their Appeal Brief, Appellants discussed the reasons why Hu *et al.* did not establish a *prima facie* case for lack of utility. The Hu *et al.* reference entitled "Analysis of Genomic and Proteomic Data using Advanced Literature Mining" (emphasis added), drew conclusions based upon statistical analysis of information obtained from published literature, and not from experimental data. The statistical analysis by Hu *et al.,* is not a reliable standard because the frequency of citation only reflect the current research interest of a molecule but not the true biological function of the molecule. It often happens that important molecules are overlooked and not published. Therefore, Appellants submit that Hu *et al.* drew their conclusions based on information which is limited. Appellant also criticizes Hu *et al.* as being limited to a specific type of breast tumor (estrogen receptor positive breast tumor). Accordingly, Hu *et al.* is not sufficient evidence to show that it is likely that PRO269 protein is not overexpressed. The Examiner does not present any meaningful arguments why these criticisms wrong. The Examiner indicates that Appellant is holding Hu to a higher standard than their own specification for statistical analysis. However, Appellants have compared the level of amplification of the PRO269 gene in normal and lung tumors and have provided information indicating a greater than 2 fold amplification. Appellants are not relying on statistical analysis of information obtained from published literature based on the current research interest of a molecule. Appellants are not holding Hu et al to a higher standard. Accordingly, Hu et al. is irrelevant to the instant discussion.

The Examiner repeatedly states that **newly cited** Dr. LaBaer allegedly made an even stronger statement that reports of mRNA or protein changes of as little as two-fold are not uncommon, and although changes of this magnitude may turn out to be important, most are attributable to disease-independent differences between the samples. (Examiner's Answer, pages 6, 15, 20, 36).

Similarly, the comments by LaBaer *et al.* entitled "Mining the literature and large

datasets" is also based on statistical analysis like Hu, and offers an automated literature mining tool termed MedGene to comprehensively summarize gene-disease relationships. As was argued in the Hu reference, "some molecules may have been underrepresented merely because they were less frequently cited or studied in literature compared to other more well-cited or studied genes." Statistical analysis using literature mining is a very useful tool to assist the researcher in their analysis but may greatly over represent or under represent certain genes and thus their conclusions may not be generally applicable. Accordingly, the statements by LaBaer are misplaced here.

The Examiner **newly cites** Chen et al. as allegedly comparing mRNA and protein expression for a cohort of genes in the same lung carcinomas. Only 17% of 165 protein spots or 21% of the genes had a significant correlation between protein and mRNA expression levels. Chen et al., allegedly clearly state that "the use of mRNA expression patterns by themselves, however, is insufficient for understanding the expression of protein products' (p. 304) and "it is not possible to predict overall protein expression levels based on average mRNA abundance in lung cancer samples" (pp.311-312). (Examiner's Answer page 6, 14, 20, 27, 31, and 35)

Nor is the analysis provided by Chen *et al.* applicable to the present application for the following reasons. First of all, Appellants note that the proteins selected for their study in Chen *et al.* were identified by staining of 2D gels. As is well known, and was noted in Haynes *et al.* for instance, there are problems with selecting proteins detectable by 2D gels: "It is apparent that without prior enrichment only a relatively small and highly selected population of *long-lived, highly expressed proteins* is observed. There are many more proteins in a given cell which are not visualized by such methods. Frequently it is the low abundance proteins that execute key regulatory functions" (page 1870, col. 1). Thus Chen *et al.*, by selecting proteins visualized by 2D gels, are likely to have excluded in their analysis many key regulatory proteins which could be candidate cancer markers.

Secondly, the manner in which the Chen data was averaged and analyzed is a vastly different manner from that of the instant specification. For example, Chen *et al.* studied

expression levels across a set of samples which included a large number of tumor samples (76) and a much smaller group of normal samples (9). The authors determined the global relationship between mRNA and corresponding protein expression using the average expression values for all 85 lung tissue samples. The authors chose an arbitrary threshold of 0.115 for the correlation to be considered significant. This resulted in negative normalized protein values in some cases and the authors concluded that it is not possible *to predict* overall protein expression based on **average mRNA abundance**. Once again, Appellants remind the Examiner that the utility standard does not require accurate prediction of protein values; only that in a majority of the proteins studied, it is more likely than not that protein levels increased when mRNA levels increased. A review of the correlation coefficient data presented in the Chen *et al.* paper indicates that, in fact, Chen teaches that 'it is more likely than not' that increased mRNA expression correlates well with increased protein expression. For instance, a review of Table 1, which lists 66 genes [the paper incorrectly states there are 69 genes listed] for which only one protein isoform is expressed, shows that 40 genes out of 66 had a positive correlation between mRNA expression and protein expression. This clearly meets the test of "more likely than not". Similarly, in Table II , 30 genes with multiple isoforms [again the paper incorrectly states there are 29] were presented. In this case, for 22 genes out of 30, at least one isoform showed a positive correlation between mRNA expression and protein expression. Furthermore, 12 genes out of 29 showed a strong positive correlation [as determined by the authors] for at least one isoform. **No genes showed a significant negative correlation**. It is not surprising that not all isoforms are positively correlated with mRNA expression. Thus, Table II also provides that it is more likely than not that protein levels will correlate with mRNA expression levels.

The Examiner cites Hanna and Mornin as showing that gene amplification does not reliably correlate with polypeptide over-expression and thus the level of polypeptide expression must be tested empirically (Examiner's Answer page 31)).

Appellants disagree. Hanna and Mornin describe HER-2/neu Breast cancer predictive testing methods which have been FDA approved: immunohistochemistry and fluorescent in situ

hybridization. While Hanna and Mornin indicate that some subsets of tumors were found lacking protein overexpression with gene amplification, Hanna and Mornin state that "in general, FISH and IHC results correlate well." (Column 2) Accordingly, it is more likely than not that protein expression with correlate with gene amplification.

The Examiner has cited Haynes *et al.*, Gygi *et al.*, Lian *et al.*, Fessler *et al.*, and Greenbaum *et al.*, as allegedly showing that increased mRNA levels do not correlate with increased protein levels in healthy tissues.

Initially, Appellants note that these analyses were done in healthy tissues and are not a comparison of an increase in mRNA expression in cancer tissues compared to healthy tissues. Accordingly, they are not relevant to the current application. Furthermore, as previously noted, Gygi *et al.*, Lian *et al.*, Fessler *et al.*, and Greenbaum *et al.*, are **newly cited** references.

The Examiner has cited Haynes *et al.* as providing evidence that there is "**no strong correlation** between polypeptide and transcript level. For some genes, equivalent mRNA levels translated into polypeptide abundances which varied more than 50-fold. Haynes et al. concluded that the polypeptide levels cannot be accurately predicted from the level of the corresponding mRNA transcript. (Examiner's Answer page 6-7, 15)

Appellants submit that it is not a legal requirement to establish a necessary or "strong" correlation between an increase in the copy number of the mRNA and protein expression levels that would correlate to the disease state, nor is it imperative to find evidence that DNA amplifications are always associated with overexpression of the gene product. As discussed above, the evidentiary standard to be used throughout *ex parte* examination of a patent application is a preponderance of the totality of the evidence under consideration. Accordingly, the question is whether it is more likely than not that a person of ordinary skill in the art would recognize a positive correlation.

Contrary to the Examiner's reading, Haynes *et al.* teaches that "there was a *general trend but no strong correlation* between protein [expression] and transcript levels" (Emphasis added). For example, in Figure 1, there is a positive correlation between mRNA and protein levels

amongst most of the 80 yeast proteins studied. In fact, very few data points deviated or scattered away from the expected normal and no data points showed a negative correlation between mRNA and protein levels (i.e. an increase in mRNA resulted in a decrease in protein levels). Appellants further note that Haynes *et al.* was studying yeast cells and not human cells. Haynes *et al.* notes that their analysis focused on the 80 most abundant proteins in the yeast lysate (page 1867). Haynes *et al.* states "since many important regulatory proteins are present only at low abundance, these would not be amenable to analysis" (page 1867). Further, Haynes *et al.* compared the protein expression levels of these naturally abundant proteins to mRNA expression levels from published SAGE frequency tables. (page 1863). Accordingly, Haynes *et al.* did not compare mRNA expression levels and protein levels in the same yeast cells. Thus the analysis by Haynes *et al.* is not applicable to the present application.

The Examiner argues that Appellants criticism of Haynes that it is pertinent only to yeast cells is misplaced. The Examiner refers to the references of Lian et al., Fessler et al., Hu et al., LaBaer, Chen et al., Hanna et al., and Greenbaum et al. as showing the same trend in other mammalian systems. (Examiner's Answer, page 19) Appellants disagree that these references show that mRNA levels do not predict protein levels for the reasons set forth herein. Appellants note that Greenbaum also studied yeast cells.

The Examiner **newly cites** Gygi *et al.* as allegedly concluding "the correlation between mRNA and protein levels was insufficient to predict protein expression levels from quantitative mRNA data. Indeed, for some genes, while the mRNA levels were of the same value, the protein levels varied by more than 20-fold.... Our results clearly delineate the technical boundaries of current approaches for quantitative analysis of protein expression and reveal that simple deduction from mRNA transcript analysis is insufficient." (Examiner's Answer page 7, 15-16, and 36)

Appellants further submit that Gygi *et al* too did not indicate that a correlation between mRNA and protein levels does <u>not</u> exist. Gygi *et al.* only state that the correlation may not be sufficient in **accurately** predicting protein level from the level of the corresponding mRNA

transcript (Emphasis added) (see page 1270, Abstract). *Accurate prediction* is not a criteria that is necessary for meeting the utility standards. In fact, contrary to the Examiner's statement, the Gygi data also indicates **a general trend** of correlation between protein [expression] and transcript levels (Emphasis added). For example, as shown in Figure 5, the mRNA abundance of **250-300** copies /cell correlates with the protein abundance of **500-1000** x 10^3 copies/cell. The mRNA abundance of **100-200** copies/cell correlates with the protein abundance of **250-500** x 10^3 copies/cell (emphasis added). Therefore, high levels of mRNA **generally** correlate with higher levels of proteins. In fact, most data points in Figure 5 did not deviate or scatter away from the general trend of correlation. Furthermore, Gygi *et al.* studied yeast cells and not the difference in expression between normal human and lung tumor cells. *Thus*, the Gygi data, meets the "more likely than not standard" and shows that a positive correlation exists between mRNA and protein. Therefore, Appellants submit that the Examiner's rejection is based on a misrepresentation of the scientific data presented in Gygi *et al.*

The Examiner **newly cites** Lian *et al.* as allegedly showing a similar lack of correlation in mammalian (mouse) cells. "The results suggest a poor correlation between mRNA expression and protein abundance, indicating that it may be difficult to extrapolate directly from individual mRNA changes to corresponding ones in protein levels". (Examiner's Answer page 7, 16, 21, and 36-37)

Regarding Lian *et al.*, Appellants submit that they only teach that protein expression may not correlate with mRNA level in differentiating myeloid cells and does not teach anything regarding such a lack of correlation for genes in general. In addition, the authors themselves admit that there are a number of problems with the data presented in this reference. At page 520 of this article, the authors explicitly express their concerns by stating that "[t]hese data must be considered with several caveats: membrane and other hydrophobic proteins and very basic proteins are not well displayed by the standard 2DE approach, and **proteins presented at low level will be missed**. In addition, to simplify MS analysis, we used a Coomassie dye stain rather than silver to visualize proteins, and this **decreased the sensitivity of detection of minor**

proteins." (Emphasis added). It is known in the art that Coomassie dye stain is a very insensitive method of measuring protein. This suggests that the authors relied on a very insensitive measurement of the proteins studied. The conclusions based on such measurements can hardly be accurate or generally applicable.

The Examiner also asserts that Fessler *et al.* (**newly cited**), who examined lipopolysaccharide-activated neutrophilins, "found a 'poor concordance between mRNA transcript and protein expression changes' in human cells." (Examiner's answer page 7, 16, and 37).

Again, as with Lian *et al.*, Fessler *et al.* only examined the expression level of **a few proteins/RNAs** in response to LPS stimulation. Additionally, the PTO has overlooked a number of limitations of the study by Fessler *et al.* For example, as admitted by Fessler *et al.*, protein identification by two-dimensional PAGE is limited to well-resolved regions of the gel, may perform less well with hydrophobic and high molecular weight proteins, and tends to select for more abundant protein species (page 31301, col. 1). Harvesting of the LPS-incubated PMNs at 4 hours may have prevented detection of earlier, **transient changes and may have thereby introduced artificial transcript-protein discordance**. Furthermore, the post-LPS incubation, pre-two-dimensional PAGE cell washes **would be expected to remove secreted proteins from further analysis**. In addition, because protein binding of Coomassie Blue has a limited dynamic range and is typically not linear throughout the range of detection, image analysis of Coomassie Blue-stained protein spots should only be consider as semi-quantitative (see page 31301, col. 1). Again, in this study, low abundance proteins were underrepresented. Therefore, Fessler's study cannot be applied to the present application.

In summary, both Fessler *et al.* and Lian *et al.* have relied on insensitive and inaccurate methods of measuring protein expression levels. The teachings of these two references cannot be relied upon to establish a *prima facie* showing of lack of utility.

The Examiner has cited Greenbaum *et al.* (**newly cited**) as:

> "caution[ing] against assuming that mRNA levels are generally correlative of protein levels. The reference teaches (p-age 117.3 2nd column) that primarily because of a limited ability to measure

protein abundances, researchers have tried to find correlations between mRNA and the limited protein expression data, in the hope that they could determine protein abundance levels from the more copious and technically easier mRNA experiments. To date, however, there have been only a handful of efforts to find correlations between mRNA and protein expression levels, most notably in human cancers and yeast cells. And, for the most part, they have reported only minimal and/or limited correlations. The reference further teaches (page 117.4, 2nd column) that there are presumably at least three reasons for the poor correlations generally reported in the literature between the level of mRNA and the level pf protein, and these may be mutually exclusive. First, there are many complicated and varied post-transcriptional mechanisms involved in turning mRNA into protein that are not yet sufficiently well defined to be able to compute protein concentrations from mRNA; second, proteins may differ substantially in their *in vivo* half lives; and/or third, there is a significant amount of error and noise in both protein and mRNA experiments that limit our ability to get a clear picture. The reverence further notes (page 117.6, page 2nd column) that to be fully able to understand the relationship between mRNA and protein abundances, the dynamic processes involved in protein synthesis and degradation have to be better understood." (Examiner's Answer, pages 7 – 8, 16-17, and 37-38).

Appellants note that Greenbaum is also comparing the expression of a number of different mRNAs and their corresponding proteins in yeast cells and not comparing the change of expression of specific mRNAs and their corresponding proteins in cancer cells versus normal cells. Accordingly, this reference is also not relevant to the issue at hand. Nevertheless, Greenbaum states that logically "we would assume that those ORFs that show a large degree of variation in their expression are controlled at the transcriptional level. The variability of the mRNA expression is indicative of the cell controlling the mRNA expression at different points of the cell cycle to achieve the resulting and desired protein. **Thus we would expect and we found a high degree of correlation (r-0.89) between the reference mRNA and protein levels for these particular ORFs: the cell has already put significant energy into dictating the final level of protein through tightly controlling the mRNA expression**. (page 117.5 1st column). Furthermore, Greenbaum states : **"we found that ORFs that have higher than average levels of ribosomal occupancy – that is that a large percentage of their cellular mRNA concentration is associated with ribosomes (being translated) – have well correlated mRNA and protein expression levels. (Figure 2)."** Therefore, contrary to the Examiner's assertion, Greenbaum does find high levels of correlation between mRNA and protein expression in yeast cells.

For the reasons given above, Appellants respectfully submit that the Examiner has not established a *prima facie* showing of lack of utility based on the new references cited in the Examiner's answer either and therefore, the Patent Office has failed to meet its initial burden of proof.

With regard to the correlation between mRNA expression and protein levels, Appellants submitted a Declaration by Dr. Polakis, principal investigator of the Tumor Antigen Project of Genentech, Inc., the assignee of the present application, to show that mRNA expression correlates well with protein levels, in general. As Dr. Polakis explains, the primary focus of the microarray project was to identify tumor cell markers useful as targets for both the diagnosis and treatment of cancer in humans.

While the proper legal standard is to show that the existence of correlation between mRNA and polypeptide levels is more likely than not, the showing of approximately 80% correlation for the molecules tested in the Polakis Declaration greatly exceed this legal standard. Based on these experimental data and his vast scientific experience of more than 20 years, Dr. Polakis states that, for human genes, increased mRNA levels typically correlate with an increase in abundance of the encoded protein. He further confirms that "it remains a central dogma in molecular biology that increased mRNA levels are predictive of corresponding increased levels of the encoded protein."

The Examiner has stated that Dr. Polakis' Declaration is allegedly not persuasive because the specification only provides information regarding PRO269 gene amplification data and does not disclose PRO269 mRNA data. Furthermore, there is allegedly strong opposing evidence showing that gene amplification is not predictive of increased mRNA levels in normal and cancerous tissues and in turn that increased mRNA levels are not predictive of increased polypeptide levels. (Examiner's Answer, page 26 – 27).

Appellants have provided reasons why the Examiner has failed to provide pertinent art which contradicts Dr. Polakis' statement that it remains a central dogma that increased mRNA levels are predictive of corresponding increased levels of the encoded protein". Furthermore, Dr.

Polakis, in his declaration, provides evidence that overexpression of mRNA correlated with overexpression of protein in 80% of the genes studied.

The Examiner states that while Dr. Polakis bases his findings on facts, the facts are allegedly not independently provided for the examiner to draw independent conclusions. For example it allegedly is not clear if any of the tumors were from lung or how highly amplified the genes were that correlated with polypeptide expression.

Appellants disagree. As Dr. Polakis explains, using microarray analysis, Genentech scientists have identified approximately 200 gene transcripts (mRNAs) that are present in human tumor cells at significantly higher levels than in corresponding normal human cells. To date, they have generated antibodies that bind to about 30 of the tumor antigen proteins expressed from these differentially expressed gene transcripts and have used these antibodies to quantitatively determine the level of production of these tumor antigen proteins in both human cancer cells and corresponding normal cells. Having compared the levels of mRNA and protein in both the tumor and normal cells analyzed, they found a very good correlation between mRNA and corresponding protein levels. Specifically, in approximately 80% of their observations they have found that increases in the level of a particular mRNA correlates with changes in the level of protein expressed from that mRNA. Accordingly, Dr. Polakis has provided the facts to enable the Examiner to draw independent conclusions.

The case law has clearly established that in considering affidavit evidence, the Examiner must consider all of the evidence of record anew.[1] "After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of the evidence with due consideration to persuasiveness of argument"[2] Furthermore, the Federal Court of Appeals held in *In re Alton*, "We are aware of no reason why

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[1] *In re Rinehart,* 531 F.2d 1084, 189 USPQ 143 (C.C.P.A. 1976) and *In re Piasecki,* 745 F.2d. 1015, 226 USPQ 881 (Fed. Cir. 1985).

[2] *In re Alton,* 37 USPQ2d 1578 (Fed. Cir 1966) at 1584 quoting *In re Oetiker,* 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992)).

opinion evidence relating to a fact issue should not be considered by an examiner"[3]. Appellants also respectfully draw the Examiner's attention to the Utility Examination Guidelines[4] which state, "Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered."

Taken together, although there are some examples in the scientific art that do not fit within the central dogma of molecular biology that there is a correlation between polypeptide and mRNA levels, these instances are exceptions rather than the rule. In the majority of amplified genes, the teachings in the art, as exemplified by Orntoft *et al.,* Hyman *et al.,* Pollack *et al.,* and the Polakis Declaration, overwhelmingly show that gene amplification influences gene expression at the mRNA and protein levels. Therefore, one of skill in the art would reasonably expect in this instance, based on the amplification data for the PRO269 gene, that the PRO269 polypeptide is concomitantly overexpressed. Thus, Appellants submit that the PRO269 polypeptides and antibodies have utility in the diagnosis of cancer and based on such a utility, one of skill in the art would know exactly how to use the antibody for diagnosis of cancer.

Accordingly, this rejection under 35 U.S.C. §101 and §112, first paragraph, should be withdrawn.

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[3] *In re Alton, supra.*

[4] Part IIB, 66 Fed. Reg. 1098 (2001).

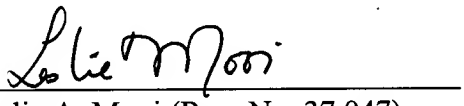
CONCLUSION

For the reasons given above, Appellants submit that the gene amplification assay disclosed in Example 92 of the specification, and the advanced state of the art in oncology, provide at least one patentable utility for the PRO269 polypeptides of Claims 44-46 and 49-52, and that one of ordinary skill in the art would understand how to use the claimed polypeptides and would have found such testing routine and not 'undue.' Therefore, Claims 44-46 and 49-52 meet the requirements of 35 U.S.C. §101 and 35 U.S.C. §112, first paragraph.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. **08-1641** (referencing Attorney's Docket No. **39780-1618 P2C33**).

Respectfully submitted,

Date: January 17, 2006

By: 
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